

The correlation between ovomucoid-derived peptides, human leucocyte antigen class II molecules and T cell receptor-complementarity determining region 3 compositions in patients with egg-white allergy

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Summary

Background Food allergies are more prevalent in children, due to the immature gastrointestinal epithelial membrane barrier allowing more proteins through the barrier and into circulation. Ovomucoid (OM) is one of the major allergens that is found in egg white.

Objective The aim of this study was to determine T cell epitopes, antigen-presenting human leucocyte antigen (HLA) class II molecules of the T cell lines (TCLs) and T cell clones (TCCs), and complementarity determining region (CDR) 3 loops of the T cell receptor (TCR) α and β chains of the TCCs specific to OM.

Methods We established TCLs and TCCs specific to OM from peripheral blood mononuclear cells (PBMCs) of four atopic patients with egg-white allergy using a mixture of a panel of overlapping synthetic peptides corresponding to the amino acid sequence of the entire OM. We identified the T cell epitopes by antigen-induced proliferative responses, antigen-presenting molecules using allogeneic PBMCs and CDR3 loops of the TCR α and β chains by cloning and sequence analysis.

Results The TCLs and TCCs responded to seven different peptides, and their antigen-presenting molecules were different from each other. Sequence analysis of the TCR α and β gene usage of the TCCs showed marked heterogeneity, and the usage of the CDR3 loop of the TCCs involved heterogeneous amino acid residues. Interestingly, TCCs 'IH3.3' and 'YT6.1' recognized the same OM peptides, and had the same TCR V β -J β gene usage. Considering that peptide motifs bind to HLA class II molecules, the electrically charged residue (positive or negative) on the CDR3 α and the CDR3 β loops of TCR of TCC may form ionic bonds with a charged residue on the HLA class II molecules-peptide complex.

Conclusions TCCs that have the same TCR gene usage were established from patients who had shown similar hypersensitivity-type, indicating that antigen recognition by a specific TCR is closely associated with the characteristics of each patient's symptoms.

Keywords complementarity determining region 3, ovomucoid, T cell clone, T cell epitope, T cell receptor
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Introduction

Food allergy has been demonstrated to play an important role in the pathogenesis of atopic dermatitis (AD), affecting approximately one-third of the children with AD [1]. It is also known that food allergies are more prevalent in children, due to the immature gastrointestinal epithelial membrane barrier allowing more proteins through the barrier and into circulation [2]. Among various food antigens, hen's egg, particularly its egg white, is one of the most common causes of food allergy in young children. Ovomucoid (OM), which constitutes 11 weight percentage of the total protein in egg white, is a highly soluble, heat-stable glycoprotein with a molecular weight of 28 000 [3, 4]. Chicken OM consists of three tandem homologous domains [5],

and antibodies specific to OM are detected frequently in the sera from patients suffering from egg allergy [6]. Some research groups have reported that OM plays a more significant role in the pathogenesis of allergic reactions to egg white than other egg white proteins [7, 8], and T cell epitopes specific to OM have recently been reported [9]. The T cell receptor (TCR) of CD4⁺ T lymphocytes recognizes immunogenic peptide sequences bound within the groove of human leucocyte antigen (HLA) class II molecules [10]. Such recognition results in a signal that is transmitted through the TCR, activates intracellular signal transduction pathways, and culminates in the proliferation and differentiation of CD4⁺ T cells into effector cells that execute many functions [11]. Proposed models of the interaction of TCR with HLA molecules orientate the diverse complementarity determining region (CDR) 3 loops above the diverse peptides and the less diverse CDR1 and CDR2 loops above the correspondingly less diverse HLA molecules [12–16]. We hypothesize that the mechanisms of allergic reactions

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originate from the recognition of the antigen presented by specific HLA class II molecules. We established OM-specific T cell lines (TCLs) and T cell clones (TCCs) from four Japanese AD patients with egg-white allergy in order to identify T cell epitopes on OM, antigen-presenting HLA class II molecules, surface-marker phenotypes, and the usage of the CDR3 loops of the TCR α and β chains of the TCCs.

Materials and methods

Patients

The diagnosis of allergy to hen's egg-white was based on clinical symptoms, antigen challenge test results and CAP-RAST [17] results to hen's egg-white and ovomucoid (Table 1). Patients TM and YN had shown symptoms of immediate type hypersensitivity such as systemic urticaria and severe cough, which occurred within 30 min after antigen challenge, and their degree of total IgE and CAP-RAST results for egg white and OM were high. Patients IH and YT had shown symptoms of delayed type hypersensitivity (DTH), such as systemic eczema, which occurred more than 24 h after antigen challenge, and their degree of total IgE and CAP-RAST for egg white and OM were low.

Synthesis of OM-derived peptides

OM-derived peptides were synthesized using the multiple method (Melbourne, Australia) based on Fmoc strategy. Peptides of 14–22 residues were overlapped by 11–15 residues, and corresponding to the entire sequences of OM. All peptides were purified by C18 reverse-phase HPLC (Gilson, Middleton, WI, USA). The primary structures of peptides used in this study are shown in Fig. 1.

Generation of antigen-specific TCLs and TCCs

OM-specific TCLs were established from peripheral blood mononuclear cells (PBMCs) from four patients with egg-white allergy. TCLs were generated by stimulating PBMC (1.5×10^5 /well) with a mixture of 23 synthetic OM-derived peptides at a final concentration of 0.1 μ M each, in the RPMI 1640 medium (Gibco, Grand Island, NY, USA) supplemented with 2 mM L-glutamine, 100 units/mL of penicillin, 100 mg/mL of streptomycin, and 10% pooled, heat-inactivated normal human male plasma in 96-well flat-bottomed culture plates (Falcon, Becton Dickinson, Lincoln Park, NJ, USA). After 7 to 9 days, irradiated (3000 cGy) autologous PBMC (1.5×10^5 /well) pulsed with OM peptide mixture (1 μ M each for 5 h), human recombinant interleukin (rIL)-2 (50 U/mL) (Genzyme, Cambridge, MA, USA) and human rIL-4 (10 U/mL) (Biosource International,

Table 1. Clinical features and HLA class II haplotypes of the subjects studied

Pt	Age	Sex	Symptom	Total IgE (IU $\ddagger$ /mL)	CAP-RAST for egg white (UA $\S$ /mL)	CAP-RAST for OM (UA $\S$ /mL)	HLA-DRB1*	HLA-DRB3*	HLA-DRB4*	HLA-DRB5*	HLA-DQA1*	HLA-DQB1*
TM	8 m	M	AD $\dagger$	2239.5	< 100	< 100	0802/0901	—/—	—/0101	—/—	0301/0301	0302/0303
YN	10 m	M	AD $\dagger$	2430.7	< 100	< 100	0406/0406	—/—	0101/0101	—/—	0301/0301	0302/0302
IH	1.1 yr	M	AD $\dagger$	30.7	< 0.34	< 0.34	0901/1403	—/0101	0101/—	—/—	0301/0501	0301/0303
YT	6 m	M	AD $\dagger$	8.4	1.2	0.6	0405/1502	—/—	0101/—	—/0102	0301/0103	0401/0601

$\dagger$ Atopic dermatitis. $\ddagger$ International unit. $\S$ Unit of allergen-specific IgE.

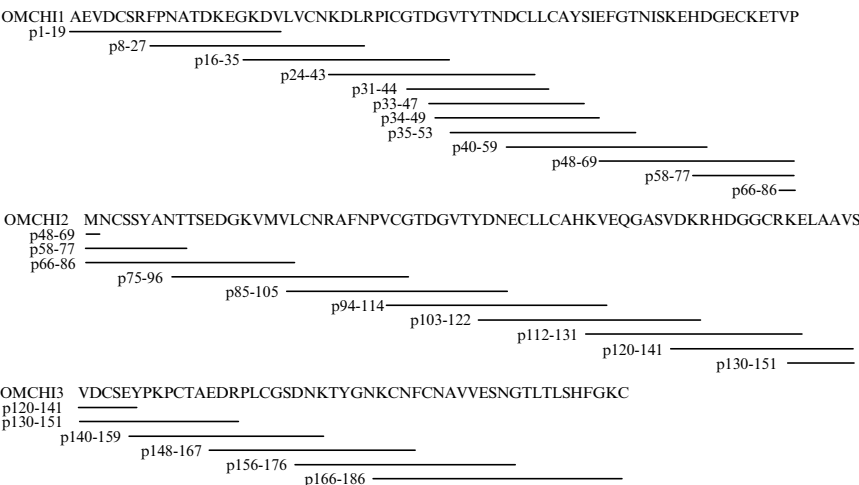


Fig. 1. Schema of the synthetic peptides. OM consists of three tandem homologous domains (OMCHI1-OMCHI3). Lines represent synthetic peptides composed of 14–22 residues overlapping each other by 11–15 residues. Numbers refer to the location of the residues in the primary structure of OM.

Camario, CA, USA) were added only to culture wells that were carrying T cell blasts and were thus maintained for another 7 days. Aliquots of growing cultures were combined as a bulk TCL for each patient, and reactivity against the overlapping peptides was determined. Cloning was done in Terasaki plates (Nunc, Roskilde, Denmark), by limiting dilution at 0.3 cells per well in the presence of irradiated (3000 cGy) autologous PBMC (3×10^4 /well) pulsed with OM peptide mixture (1 μ M), human rIL-2 (50 U/mL) and human rIL-4 (10 U/mL), in the same medium described above. Growing microcultures (approximately 5% of all culture wells) were then expanded at weekly intervals, first in a 96-well plate and then in a 24-well plate by feeding irradiated feeder cells (1.5×10^6 /well) pulsed with OM peptide mixture (1 μ M), in the presence of rIL-2 and rIL-4.

Antigen-induced proliferative responses of the TCLs and TCCs

Antigen-induced proliferation of the TCLs and TCCs was assayed by culturing the T cells (3×10^4 /well) in 96-well flat-bottomed culture plates in the presence of the soluble OM peptide mixture (1 μ M each), respective overlapping peptides (1 μ M), or OM protein (2.5 or 25 mg/mL) and 3000 cGy-irradiated autologous PBMC (1.5×10^5 /well). Cells were cultured for 72 h, in the presence of 1 mCi/well of [^{3} H]TdR during the final 16-h period, and the incorporated radioactivity was measured by liquid scintillation counting. To determine restriction molecules for antigen presentation, the TCLs and TCCs were cultured with irradiated autologous PBMC, with or without anti-HLA class II monoclonal antibodies (mAbs) HU-4 (anti-HLA-DRB1 + DRB5 IgG2a, monomorphic) [18, 19], L243 (anti-HLA-DRB1 + DRB4 IgG2a, monomorphic, reactivity to DRB3 and DRB5 yet to be determined) [20], HU-11 (anti-HLA-DQ4 + DQ5 + DQ6 IgG2a) [21], HU-18 (anti-HLA-DQ7 + DQ8 + DQ9 IgG2a) [22], HU-46 (anti-HLA-DQ4 IgG2a) [21] and B7/21 (anti-HLA-DP IgG1, monomorphic) [23]. The percentage inhibition was calculated using the following formula: $\{1 - (\text{Value obtained with a peptide and a mAb} - \text{that with medium alone}) / (\text{Value obtained with a peptide and without a mAb} - \text{that with medium alone})\} \times 100$. Other experiments were done in which PBMC of two donors with one haplotype-shared and one donor with irrelevant HLA haplotypes were used as antigen presenting cell (APC). In this experiment, allogeneic PBMC (3×10^5 /well) were cultured in plates with peptides (1 μ M each) in the culture medium, for 2 h at 37 °C, then excess peptides and non-adherent cells were removed by gently washing the plates three times with RPMI 1640 media containing 3% human serum. The remaining adherent cells were irradiated at 3000 cGy and used as APC.

HLA typing

HLA class II (DR, DQ) alleles were determined by hybridization of HLA-DR, DQ genes amplified by polymerase chain reaction with sequence-specific oligonucleotide probes distributed in the 11th International Histocompatibility Workshop, as described elsewhere [24]. The nomenclature of the HLA-DR, DQ alleles was according to the WHO Nomenclature Committee for factors of the HLA system [25].

Flow cytometric analysis

Fluorescein-isothiocyanate (FITC)-conjugated mAbs anti-Leu2a/CD8, anti-TCR- α/β -1-WT31 (Becton Dickinson, San Jose, CA, USA), anti-TCR- γ/δ I (Endogen, Woburn, MA, USA), phycoerythrin (PE)-labelled anti-Leu4/CD3 and anti-Leu3a/CD4 (Becton Dickinson) were used to analyse the phenotype of our TCCs by double colour staining. TCCs were washed with PBS, and 10^5 cells were incubated and double-stained with CD4 (PE conjugated)/CD8 (FITC conjugated) or CD3 (PE conjugated)/anti-TCR- α/β -1 WT31 (FITC conjugated) or CD3 (PE conjugated)/anti-TCR- γ/δ (FITC conjugated). Stained cells were analysed using a FACScan instrument (Becton Dickinson). Forward scatter threshold was set to exclude only debris in the preparation.

First-strand-cDNA synthesis and PCR amplification

The total RNA was extracted from TCCs using the single-step guanidinium-phenol-chloroform extraction method [26]. Single-stranded-cDNA synthesis was carried out using 1.2 to 1.8 μ g of total RNA with oligo (dT) and avian myeloblastosis virus reverse transcriptase. TCR α and β cDNAs were directly subjected to DNA amplification by polymerase chain reaction (PCR) using Amplitaq DNA polymerase (Perkin Elmer, Norwalk, CT, USA) with oligonucleotide primers complementary to TCR V and C region sequences [27] at a final concentration of 0.25 μ M. Reaction mixtures were incubated in a DNA thermal cycler (model No. PJ1000, Perkin Elmer) under the following conditions: denaturation at 93 °C for 1 min, annealing at 55 °C for 30 s and extension at 72 °C for 1 min, with a further 5-s extension after each cycle.

Cloning and sequence analysis of TCR α and β chains

The PCR reaction products were cloned into the TA cloning vector pCR IITM (Invitrogen Corporation, San Diego, CA, USA) and sequenced by the enzymatic chain termination method [28], using a sequence version 2.0 kit (United States Biochemical Corporation, Cleveland, OH, USA). For each of the TCR α and β chains studied, three independent pCR IITM cloning vectors were sequenced to identify possible sequence errors caused by nucleotide misincorporation by the Taq polymerase. Sequences were compared with gene data bank entries (GenBank, Los Alamos, NM, USA; EMBL, Heidelberg, Germany) and assigned to available published TCR V and J segments [29–32]. Virtually all TCR V transcripts have the amino acid sequence Y-(L/F)-C-A (α chain) or Y-(L/F)-C-A with two additional serine residues in most cases (β chain) at the 3' end of the V gene segment and the motif F-G-X-G-T within the J segment [33]. The CDR3 length was taken as the number of amino acids between the last germline-encoded residue of the V segment and the conserved phenylalanine residue.

Results

Polyclonal TCLs specific to OM

TCLs specific to OM were established from PBMCs of four patients with hen's egg white allergy. The cell lines were tested for their proliferative response to a panel of overlapping synthetic peptides corresponding to the amino acid sequence of the entire OM. TCL established from patient TM proliferated in

the presence of OM peptides 8–27, 35–53, 40–59, 85–105, 103–122, 156–176 and 166–186 (Fig. 2a). TCL from YN proliferated in the presence of OM peptides 40–59, 48–69, 75–96, 120–141 and 130–151. TCL from IH proliferated in the presence of OM peptides 75–96, 85–105, 120–141, 130–151 and 166–186. TCL from YT proliferated in the presence of OM peptides 120–141 and 130–151.

Antigen-presenting HLA molecules of TCLs specific to OM

TCLs from patients TM and YT were inhibited by HU4 (anti-DRB1 + 5 mAb, 41.7% and 7.6%, respectively) and L243 (anti-DRB1 + 4 mAb, 61.0% and 99.5%, respectively). TCL from YN was inhibited by HU11 (anti-DQ4 + DQ5 + DQ6

mAb, 17.0%) and HU18 (anti-DQ7 + DQ8 + DQ9 mAb, 14.3%), and TCL from IH was inhibited by L243 (anti-DRB1 + 4 mAb, 87.9%) (data not shown).

TCCs specific to OM

TCCs specific to OM were established from patients TM, YN, IH and YT; each was cloned from the TCLs mentioned above (Fig. 2b). TCC 'TM1.3' recognized OM peptides 35–53 and 40–59, whereas TCC 'TM1.4' recognized OM peptide 166–186. TCC 'YN1.5' recognized OM peptides 40–59 and 48–69, and TCC 'IH3.3' and 'YT6.1' recognized OM peptides 120–141 and 130–151.

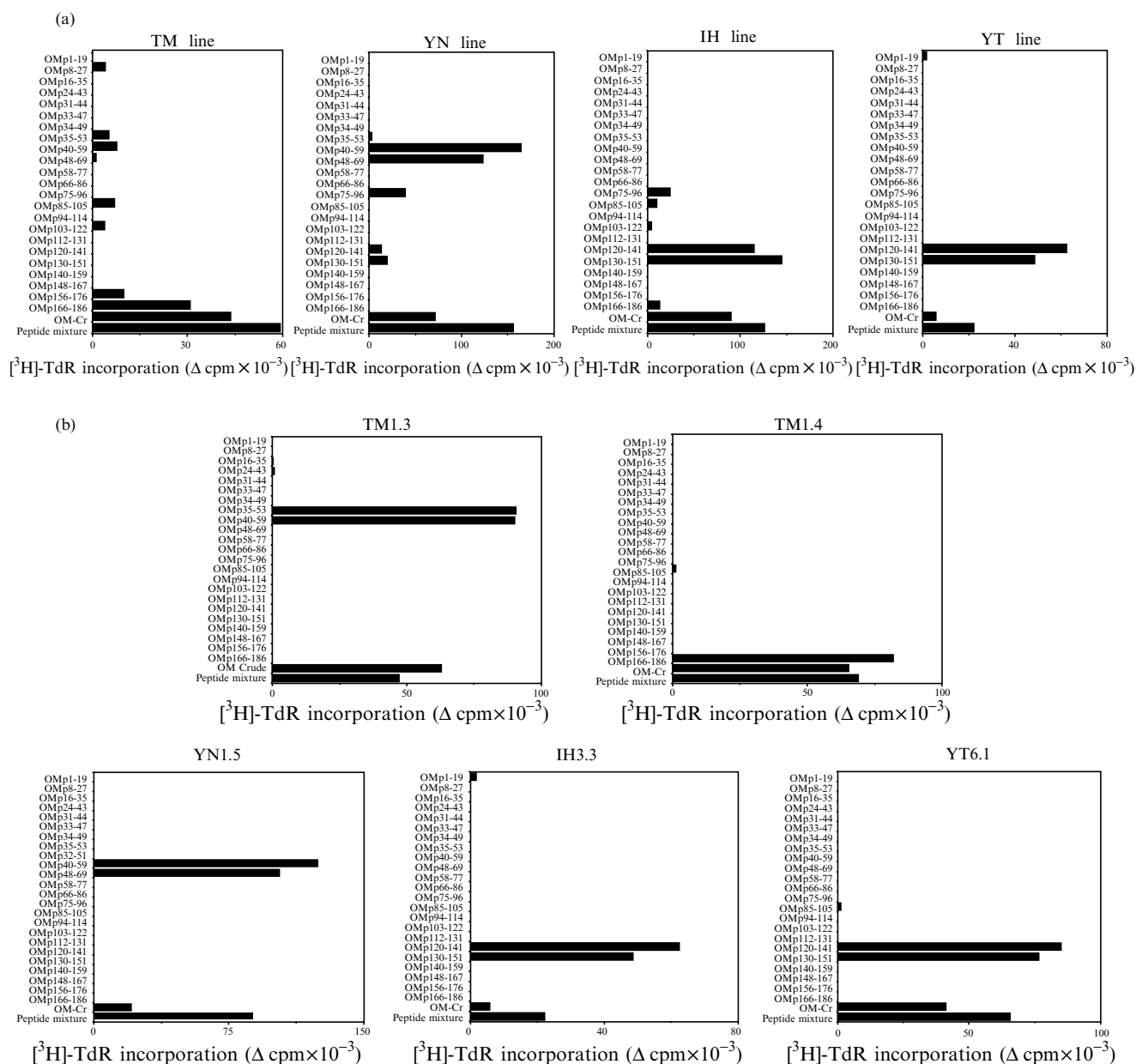


Fig. 2. Proliferative responses of four short-term T cell lines (a) and T cell clones (b) to 11- to 15-residue-overlapped synthetic peptides derived from OM. Peptides are designated by the amino acid residue numbers of the amino- and carboxyl-termini. Values shown are the mean cpm of duplicate cultures after subtraction of the mean cpm obtained from cultures without peptides. The standard error (SE) of duplicate cultures was < 20%.

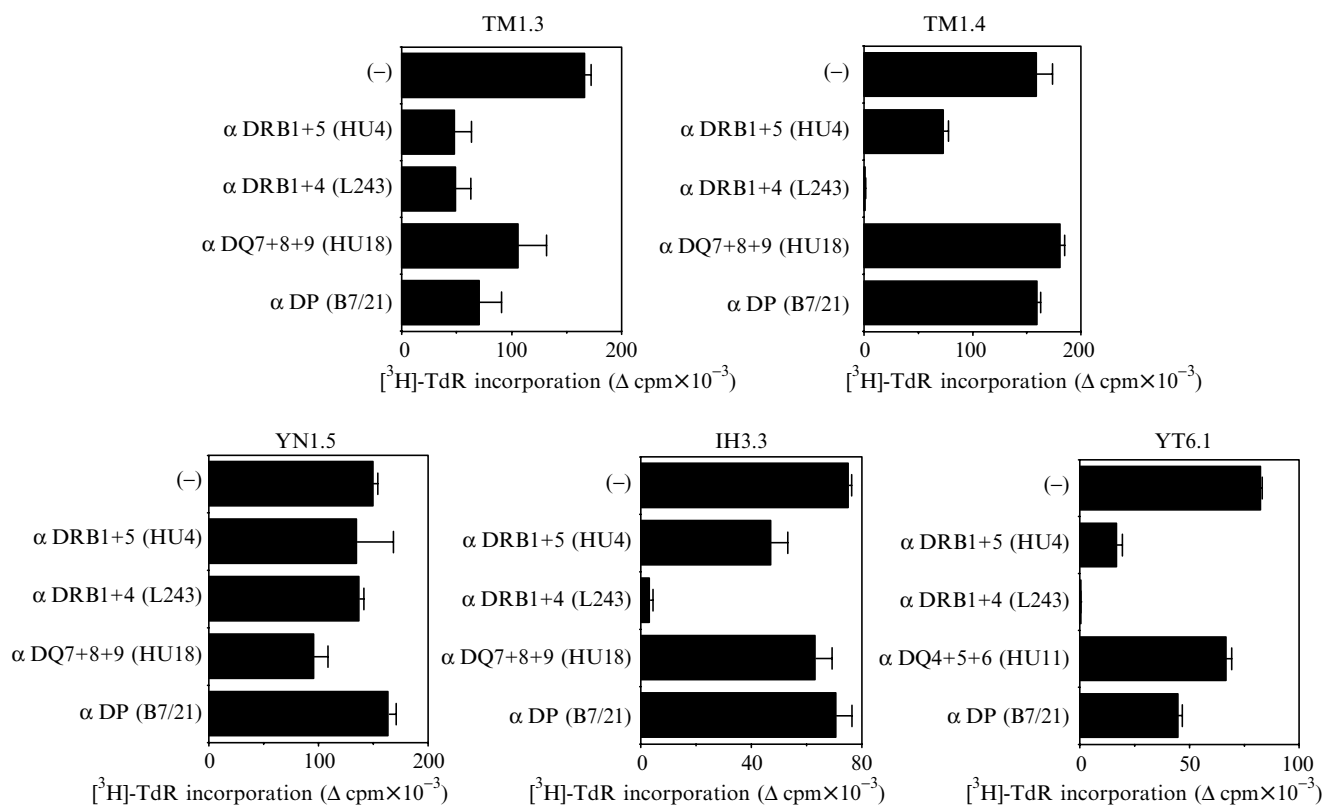


Fig. 3. Proliferation inhibition of five short-term T cell clones using anti-HLA class II mAbs. T cells (3×10^4 /well) were incubated with HU4 (anti-HLA-DRB1 + DRB5 mAb), L243 (anti-HLA-DRB1 + DRB4 mAb), HU11 (anti-HLA-DQ4 + DQ5 + DQ6 mAb), HU18 (anti-HLA-DQ7 + DQ8 + DQ9 mAb), HU46 (anti-HLA-DQ4 mAb) and B7/21 (anti-HLA-DP mAb), and irradiated autologous PBMCs (1.5×10^7 /well), with or without OM peptides ($1 \mu\text{M}$ each). Results are expressed as the geometric mean of triplicate determinations $\pm$ standard error. All mAbs were tested at a final dilution of 1:200.

Antigen-presenting HLA molecules of TCCs specific to OM
TCCs 'TM1.3', 'TM1.4' and 'YT6.1' were inhibited by HU4 (anti-DRB1 + 5 mAb, 71.3%, 54.0% and 7.6%, respectively) and L243 (anti-DRB1 + 4 mAb, 70.5%, 99.4% and 99.5%, respectively). TCC 'YN1.5' was inhibited by HU18 (anti-DQ7 + DQ8 + DQ9 mAb, 35.7%), and 'IH3.3' was inhibited by HU4 (anti-DRB1 + 5 mAb, 37.3%) and L243 (anti-DRB1 + 4 mAb, 95.6%) (Fig. 3).

HLA recognition patterns of TCCs specific to OM

TCCs 'TM1.3' and 'TM1.4' proliferated in response to OM peptides presented by allogeneic PBMCs sharing HLA-DRB1*0901. TCC 'YN1.5' proliferated in response to OM peptides presented by allogeneic PBMCs sharing HLA-DQB1*0302, TCC 'IH3.3', HLA-DRB4*0101, and TCC 'YT6.1', HLA-DRB1*0405 (Fig. 4).

TCR α and β gene usage and comparison of CDR3 loop of TCCs specific to OM

Sequence analysis of the TCR α and β gene usage of the TCCs specific to OM revealed marked heterogeneity (Fig. 5). TCCs 'TM1.3' and 'TM1.4' were cloned from the same TCL from patient TM; nevertheless, their TCR α and β gene usages were different (TCRAV19S1 and AJ48, TCRAV8S1 and AJ41; TCRBV22S1 and BJ1S6, TCRBV3S1 and BJ2S2, respectively). Interestingly, TCCs 'YN1.5', 'IH3.3' and 'YT6.1' have the same TCR V β gene usage (TCRBV2S1). TCCs 'IH3.3' and 'YT6.1'

also have the same TCR J β gene usage (TCRBJ1S1). Moreover, the CDR3 loop of the TCCs specific to OM was composed of heterogeneous amino acid residues.

Surface-marker phenotypes

All the TCLs and TCCs were of CD3⁺, CD4⁺, CD8⁻, $\alpha\beta$ TCR⁺, $\gamma\delta$ TCR⁻ phenotype (data not shown).

Discussion

Our previous study showed that seven major T cell epitopes were identified by TCCs specific to β -lactoglobulin [34]; in this study, several epitopes were identified by five OM-specific TCCs. Four T cell epitopes specific to OM (OM41-56, OM71-84, OM131-144 and OM171-186) have recently been reported [9]. Our six OM peptides (35-53, 40-59, 48-69, 120-141, 130-151, 166-186) have three (OM41-56, OM131-144 and OM171-186) of their four epitopes overlapping, indicating that T cell epitopes specific to OM are identical regardless of species. Even though Zhang et al. [35] reported that there were significantly more activities of IgG and IgE binding to the third domain of OM than to the first and second domains using egg-allergy patients' sera, Cooke et al. [36] mentioned that IgE- and IgG-binding epitopes specific to OM existed in all OM domains. Our investigation also showed that T cell epitopes specific to OM existed in all OM domains. Our previous report showed that the TCCs specific to β -lactoglobulin tend to associate with

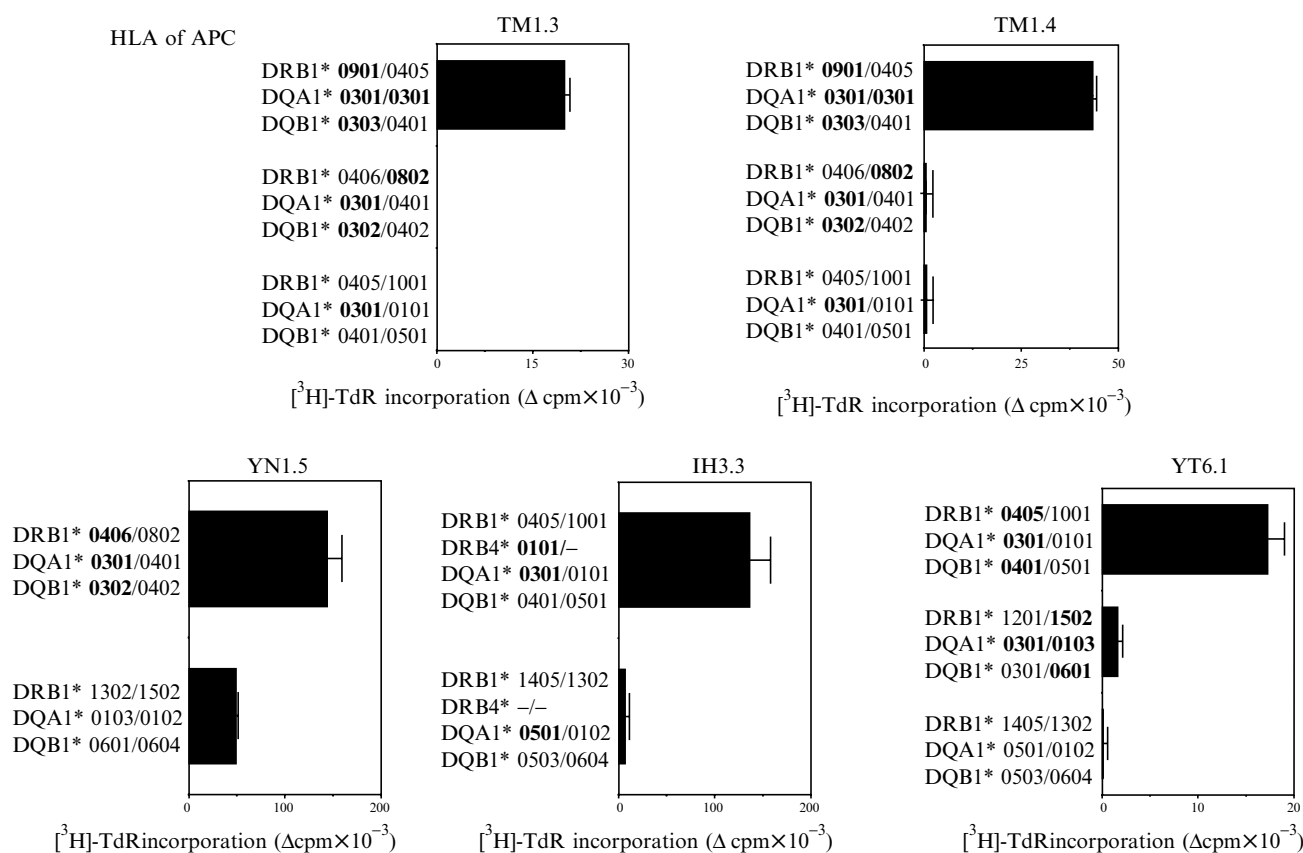


Fig. 4. Antigen presentation to our T cell clones by allogeneic PBMCs. Allogeneic PBMCs (3×10^5 /well) were cultured in a 96-well plate with $1 \mu\text{M}$ of the OM peptide mixture for 2 h at 37°C ; excess peptides and non-adherent cells were removed by gently washing the plate three times with the RPMI 1640 medium containing 3% human serum. The remaining adherent cells were irradiated at 3000 cGy and used as APC. T cells (3×10^4 /well) were then added to the culture plate. Shared HLA haplotypes are indicated in bold letters. Results are expressed as the geometric mean of triplicate determinations $\pm$ standard error.

the HLA-peptide complex presented by HLA-DRB1*0405 [37]. The antigen-presenting molecules used by TCCs specific to OM were different for each TCC.

Although the diversity-generating mechanisms for antibodies and TCR are very similar, there is much more diversity in TCR J segments and N additions at CDR3 [12], and apparently less variation at CDR1 and CDR2 [38]. Sequence analysis of the TCR α and β gene usage of our TCCs specific to OM showed marked heterogeneity. 'TM1.3' and 'TM1.4' were cloned from the same TCL from patient TM; nevertheless, they recognized different peptides, and their usage of the CDR3 loops of the TCR α and β chains was different. These peptides were presented by the same HLA molecule (DRB1*0901). Only three to five amino acids within a peptide bound to the groove of HLA molecules face outwards into the solvent for potentially direct contacts with TCR amino acids [39–41]. Similarly, although many amino acids constitute the framework of the TCR α and β chains, only a few amino acids have been confirmed to directly contact amino acids [42–47].

As shown in Fig. 5, each of the CDR3 α and CDR3 β loops of TCCs has the positively charged residues 'Q', 'K', 'N' and 'R' and the negatively charged residues 'E' and 'D'. Considering that peptide motifs bind to HLA class II molecules as reported

previously [48–50], the electrically charged residue (positive or negative) on the CDR3 α and the CDR3 β loops of TCR of TCC may form ionic bonds with a charged residue on the HLA class II molecule-peptide complex. Our previous report also showed that the negatively charged residue 'E' existed in the HLA-DRB1*0405- β -lactoglobulin peptide complex, and the positively charged residue 'K' existed in the CDR3 α loop [37].

The antigen-presenting molecules of TCCs 'IH3.3' and 'YT6.1' from patients IH and YT were different, but the TCCs recognized the same OM peptides 120–141 and 130–151. Although the CDR3 loop of TCCs 'YN1.5', 'IH3.3' and 'YT6.1' was composed of heterogeneous amino acid residues, the TCCs have the same TCR V β gene usage, and 'IH3.3' and 'YT6.1' also have the same TCR J β gene usage. Moreover, it is an attractive finding that patients IH and YT had shown symptoms of DTH. These data suggest that antigen recognition by a specific TCR is closely associated with the characteristics of each patient's symptoms.

Further experiments investigating other food antigens will clarify the correlation among antigens, antigen-presenting molecules and TCR, and assess immunopathologies of patients with AD. Furthermore, these studies will lead to the development of therapeutic approaches for AD.

(a)

T cell clone	TCR gene segment	TCRAV	CDR3	TCRAJ
TM1.3	AV19S1 AJ48	CCCAGAGACTCTGCCGTCTACATCTGTGCTGTC P R D S A V Y I C A V	CTCTTTCAGGGAGCCAGAGCTGGTA L F Q G A Q K L V	TTTGCCAAGGAACAGGCTGACTATCAAC F G Q G T R L T I N
TM1.4	AV8S1 AJ41	CCTGAAGACTCGGCTGTCTACTTCTGTGCAGCA P E D S A V Y F C A A	AGTGGGGTTCGGGTATGCACTCAAC S G A S G Y A L N	TTGGCAAAGGACCTCGCTGTTGGTCACA F G K G T S L L V T
YN1.5	AV4 AJ42	CTGAGAGACTGCTGTGTACTATTGCATCGTC L R D T A V Y Y C I V	AGGGGAGGAAGCAAGGAAATCTCATC R G G S Q G N L I	TTTGAAAAGGCACTAACTCTCTGTATAA F G K G T K L S V K
IH3.3	AV2S1 AJ14	CTCAGTGATTAGCCACCTACCTCTGTGTGGTG L S D S A T Y L C V V	AACGTAGGGAGTGCTTCAAGATAATC N V G S A S K I I	TTTGGATCAGGGACCACTCAGCATCCGG F G S G T R L S I R
YT6.1	AV7 AJ37	ACTGGGACTCAGCCACCTACCTCTGTGCC T G D S A T Y L C A	CCCGCCGAGTGAGCAACAGGCAAACTAATC P G G V S N T G K L I	TTTGGGCAAGGGCAACTTACAAGTAA F G Q G T T L Q V K

(b)

T cell clone	TCR gene segment	TCRBV	CDR3	TCRBJ
TM1.3	BV22S1 BJ1S6	CTGGAGGACTCAGCCATGTACTTCTGTGCCAGC L E D S A M Y F C A S	ACCCGCATTCGTGGGGTTCGATGGCTCACAC T R I R G G S D G S H	TTGGTTCGGGGACAGGTTAACCGTTGTA F G S G T R L T V V
TM1.4	BV3S1 BJ2S2	ACCAACAGACATCTATGTACTCTGTGCC T N Q T S M Y L C A	ACCTATAGCGGGAGCTGTTT T Y S G E L F	TTTGAGAAGGCTCTAGGCTGACCGTACTG F G E G S R L T V L
YN1.5	BV2S1 BJ1S5	CCTGAAGACAGCAGCTTCTACATCTGCAGTGCG P E D S S F Y I C S A	TGGGACTCCGATCAGCCCCAGCAT W D S D Q P Q H	TTTGGTGATGGGACTCGACTCTCCATCCTA F G D G T R L S I L
IH3.3	BV2S1 BJ1S1	CCTGAAGACAGCAGCTTCTACATCTGCAGTGCT P E D S S F Y I C S A	AGAGCGGGGGGATGAACACTGAAGCTTTC R A G G M N T E A F	TTTGACAAGGCACCACTCAGAGTTGTA F G Q G T R L T V V
YT6.1	BV2S1 BJ1S1	CCTGAAGACAGCAGCTTCTACATCTGCAGTGCT P E D S S F Y I C S A	AGTATGCGACAGGGGAGTGGTAGCTTTC S M R Q G A V V A F	TTTGACAAGGCACCACTCAGAGTTGTA F G Q G T R L T V V

Fig. 5. TCR α (a) and β (b) gene usages and CDR3 loops of the TCCs. Nucleotide and deduced amino acid sequences of junctional regions of the TCR of T cell clones specific to OM are shown. The underlined CDR3 sequences are charged residues.

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